

Amendment to the claims:

Claims 6-7, 9, 12 and 22 have been canceled. Cancellation of claims 6-7, 9, 12 and 22 is without prejudice, without intent to abandon any original claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants reserve the right to file one or more continuing applications containing these canceled claims.

Claims 1-5, 8, 10-11, 13-17, 20-21 and 23-27 have been amended. In particular, claim 1 has been amended to specify that:

- (i) The preamble limitation that the encapsulation vesicle be “*for an assay*” has been *canceled*. Removal of this preamble limitation is appropriate since Applicants have invented a patentable composition of matter. Limiting the broadest composition claim to a specific use is therefore neither necessary nor appropriate.
- (ii) The surface coating includes a “*fluorescent donor molecule*”. Support for this amendment can be found in canceled claim 7; page 6, line 17 to page 8, line 4; etc.
- (iii) The fluorescent donor molecule is “*absorbed on, absorbed within or covalently attached*” to the surface of the matrix. Support for this amendment can be found on page 4, lines 10-13 and lines 22-24; page 6, lines 13-16; etc.
- (iv) Upon irradiation the fluorescent donor molecule emits fluorescence that is “*at least partially transmitted through the surface coating*”. Support for this amendment can be found in claims 15 and 16; page 2, lines 21-24; page 4, lines 14-15 and 24-29; page 8, lines 20-22; page 9, lines 1-3; Figures 4A-4D; etc.
- (v) The protection layer “*reduces collisional quenching of the fluorescence*”. Support for this amendment can be found on page 2, lines 24-26; page 4, lines 24-26; page 7, lines 15-16; page 8, lines 14-24; page 10, lines 20-24; etc.
- (vi) The “*ligand attached to the protection layer*” limitation has been *canceled* and placed in new dependent claim 32. Removal of this limitation is supported by the original disclosure, e.g., on page 5 in Examples 1 and 2; Figure 1; page 6, lines 1-3; page 8, line 14 to page 9, line 9; etc. In particular, it is readily apparent from the teachings of the specification that attachment of a ligand to the protection layer is an optional embodiment of the invention.

Amendments to claims 2-5, 8, 10-11, 13-17, 20-21 and 23-27 are also supported by the specification and claims as originally filed, e.g., as follows:

Amended claim(s)	Support
2-3	pg. 4, ln. 4-7; pg. 6, ln. 7-13
4-5	claim 8; pg. 6, ln. 17 – pg. 8, ln. 4
8	pg. 6, ln. 17 – pg. 8, ln. 4
10	claim 9; pg. 6, ln. 26 – pg. 7, ln. 5
11	pg. 6, ln. 24-25
13	pg. 7, ln. 17 – pg. 8, ln. 4
14	pg. 7, ln. 15-16
15-16	pg. 4, ln. 14-15; pg. 9, ln. 1-3
17	pg. 5, examples 1-2
20	pg. 9, ln. 9-18
21	title; pg. 1, ln. 8-11; claims 1 & 21; pg. 10-13, examples 1-3
23	pg. 3, ln. 28 – pg. 4, ln. 3; pg. 8, ln. 8-10
24	pg. 3, ln. 31-33; pg. 8, ln. 11-13
25	pg. 4, ln. 18-21
26	fig. 4b; example 2, pg. 11-12
27	pg. 4, ln. 18-21; fig. 4d; example 3, pg. 12-13

Claims 32-49 have been added. Applicants submit that no new matter has been presented with these new claims and that support can be found throughout the specification and claims as originally filed, e.g., as follows:

New claim(s)	Support
32	fig. 4a; example 1, pg. 10-11
33	fig. 4c; example 3, pg. 12-13
34-35	fig. 4d; example 3, pg. 12-13
36	claim 29; pg. 13, ln. 15-19
37	claim 1
38	pg. 4, ln. 18-21

39	claim 17; pg. 9, ln. 3-4
40	pg. 3, ln. 32-33; pg. 8, ln. 10-11
41	claim 30; pg. 13, ln. 20-25
42	fig. 2; pg. 9, ln. 23-31
43	pg. 3, ln. 32-33; pg. 8, ln. 10-11
44	pg. 3, ln. 31-33; pg. 8, ln. 11-13
45	claim 30; pg. 13, ln. 20-25
46	pg. 3, ln. 28 – pg. 4, ln. 3; pg. 8, ln. 8-10
47	pg. 2, ln. 24-26; pg. 4, ln. 24-26; pg. 7, ln. 15-16; pg. 8, ln. 14-24; pg. 10, ln. 20-24
48	claim 9; pg. 6, ln. 26 – pg. 7, ln. 5
49	claim 12; pg. 7, ln. 5-12

As required, attached hereto as **Appendix B** is a marked-up version of the changes made to the claims by the present Amendment. For the Examiner's convenience, also attached hereto is an **Appendix C** showing all pending claims as amended remaining in this application.

Rejections under 35 U.S.C. §112:

Claims 1-27 stand rejected for failing to comply with the requirements of 35 U.S.C. §112.

With respect to the “*for an assay*” language in original claim 1, Applicants note that this language has been canceled from claim 1 and, as suggested by the Examiner, replaced with:

- (i) “*for use in a fluorescence energy transfer immunoassay*” in dependent claim 21 (further specifying a sandwich or competitive binding immunoassay in claims 26 and 32 that depend from claim 21).
- (ii) “*for use in a DNA or RNA fluorescence energy transfer hybridization assay*” in dependent claim 33.
- (iii) “*for use in a fluorescence energy transfer binding assay between a ligand and a receptor*” in dependent claim 34 (further specifying binding between an aptamer and a protein in claim 35 that depends from claim 34).

Applicants respectfully submit that these fluorescence energy transfer assays are fully described and enabled by the teachings of the present application.

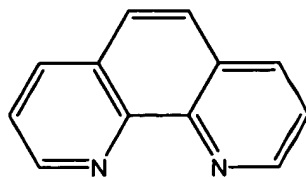
With respect to the “*matrix*” and “*surface coating*” language in original claim 1, Applicants have amended claim 1 to specify that the surface coating includes a fluorescent donor molecule that is either absorbed within the matrix, absorbed on the surface of the matrix, or covalently attached to the surface of the matrix. Applicants respectfully submit that as amended these limitations are clearly described and enabled by the teachings of the present application.

With respect to the “*protection layer*” and “*quencher*” language in original claim 1, Applicants have amended claim 1 to specify that when exposed to irradiation, the fluorescent donor molecule emits fluorescence that is at least partially transmitted through the protection layer. In addition, claim 1 has been amended to explicitly specify that the protection layer acts to reduce collisional fluorescence quenching. Applicants respectfully submit that as amended these limitation are clearly described and enabled by the teachings of the present application.

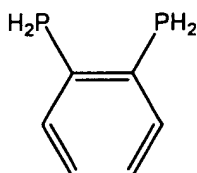
With respect to the “*ligand*” language in original claim 1 (now canceled and placed in dependent claim 37), the Examiner states that all types of “ligand” are not enabled since the claims lack a requirement that the “ligand” have a “receptor”. Applicants are puzzled by this rejection and respectfully submits that is it *inherent* that a ligand has a receptor – specifying such would be redundant. Indeed, it is well accepted that a ligand is a “molecule that binds another molecule”, i.e., that a ligand must have a receptor to be called a “ligand”. This common art definition is even explicitly disclosed in the specification (see page 4, lines 18-19). Withdrawal of this rejection is requested.

There appears to be some confusion regarding the language used by Applicants to describe certain embodiments of the nitrogen and phosphorous ligands that are present in the fluorescent donor molecules of claims 9 and 12.

With regards to the language “*aryl(s) leading to a non-substituted or substituted phenanthroline*” in claims 9 and 12, it was Applicants’ intention to cover the taught embodiments in which one or more non-substituted and/or substituted phenanthrolines were used as ligands in a fluorescent donor molecule (e.g., see page 7, lines 1-3). The structure of non-substituted phenanthroline is:



With regards to the language “*aryl leading to a non-substituted or substituted ortho-aromatic phosphines*” in claim 12, it was Applicants’ intention to cover the taught embodiments in which non-substituted and/or substituted ortho-aromatic phosphines were used as ligands in a fluorescent donor molecule. The structure of non-substituted ortho-aromatic phosphine is:



Applicants have canceled claims 9 and 12 and has rewritten these as new claims 48 and 49 to clarify the intended scope of the claims. Please note that hydrogen atoms that were inadvertently omitted from the phosphine structures in the originally filed application (i.e., PH<sub>2</sub>) have been added to the structures of claim 49. This correction adds no new matter since a skilled person would readily recognize that their omission was an obvious clerical error. Applicants respectfully submit that new claims 48 and 49 comply fully with the requirements of 35 U.S.C. §112, second paragraph.

The Examiner has also raised a number of minor issues under 35 U.S.C. §112, second paragraph that are now considered in turn:

**Item 6b** - claim 1 (that now includes the limitation of claim 7) refers to a *fluorescent* donor molecule.

**Item 6d** - claim 1 now refers to a fluorescent donor molecule.

**Item 6e** - claim 22 has been canceled. In addition, claim 20 specifies that “said ligand comprises an acceptor molecule *that is capable of absorbing fluorescence that has been emitted from said fluorescent donor molecule.*”

**Item 6f** - claim 23 has been amended to read “wherein *an* absorption band of said acceptor molecule overlaps with *an* emission band of said fluorescent donor molecule.”

**Item 6g** - claim 24 has been amended to depend from claim 20 that refers to an acceptor molecule.

**Item 6h** - claim 27 has been amended to read “wherein said *ligand* is selected from [...]”. In addition, claim 27 has been amended to depend from claim 37 that refers to a ligand.

Applicants respectfully request that these rejections be withdrawn.

Rejections under 35 U.S.C. §102:

Claims 1, 4, 5, 20, 21, and 23-27 stand rejected under 35 U.S.C. §102 as being anticipated by one or both of Hainfeld et al. (U.S. Patent No. 5,521,289) and Abbott et al. (PCT Publication No. WO 00/32044). Applicants note that original claim 7 is not anticipated by either of these two references. Original claim 7 specifies that the inventive encapsulation vesicle includes a fluorescent donor molecule within a surface coating. Omission of claim 7 from the novelty rejection is appropriate since neither one of Hainfeld et al. or Abbott et al. teaches or suggests a composition that includes a fluorescent donor molecule within a “surface coating”. As noted above, the present Amendment incorporates the limitations of claim 7 into independent claim 1 (and hence also into claims 4, 5, 20, 21, and 23-27 that depend from claim 1). Accordingly, by definition, the rejection of claims 1, 4, 5, 20, 21, and 23-27 under 35 U.S.C. §102 in view of Hainfeld et al. and/or Abbott et al. is moot. Withdrawal is respectfully requested.

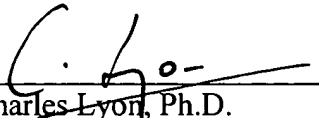
**Conclusion**

A check is enclosed in the amount of \$234 to cover the extra claim fee as follows:

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDITIONAL FEES
TOTAL CLAIMS	44	MINUS	31	= 13	\$18	\$234
INDEP. CLAIMS	3	MINUS	3	= 0	\$84	0
FIRST PRESENTATION OF A MULTIPLE DEPENDENT CLAIM?				NO	\$280	0
TOTAL EXTRA CLAIM FEE FOR THIS AMENDMENT						\$234

Please charge any additional fees that may be associated with this filing, or credit any overpayment, to our Deposit Account No. 03-1721. Applicants would like to thank Examiner Ceperley for her time and consideration of this case.

Respectfully Submitted,

  
Charles Lyon, Ph.D.  
Agent for Applicant  
Limited Recognition Under 37 CFR §10.9(b)

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Dated: February 28, 2003

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Linda M. Amato

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Linda M. Amato

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## **APPENDIX A**

### **SPECIFICATION WITH MARKINGS TO SHOW CHANGES MADE**

The paragraph that begins on line 17 of page 2 of the specification has been amended as follows:

The invention [is] provides a composition of matter and method of using the same for immunoassays. The encapsulation vesicle comprises a matrix, a surface coating with an organo-metallic complex and a transparent protection layer. The protection layer is capable of modification by addition of biomolecules to the exterior surface. The biomolecules may comprise one or more acceptor molecules. The proximity of the bound biomolecules to the protection layer allows for energy transfer from donor molecules that are inside of the transparent protection layer to the acceptor molecules that are outside the transparent protection layer. The transparent protection layer acts to diminish the effects of collisional quenching by small molecules such as oxygen to the donor molecules. The method includes a number of novel immunoassays that utilizes energy transfer between one or more donor and acceptor molecules.



## APPENDIX B

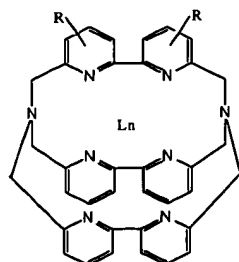
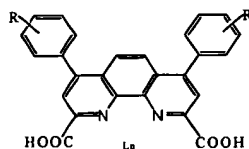
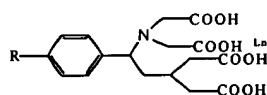
### CLAIMS WITH MARKINGS TO SHOW CHANGES MADE

Claims 6-7, 9, 12 and 22 have been **canceled** and claims 32-49 have been **added**.

Claims 1-5, 8, 10-11, 13-17, 20-21 and 23-27 have been **amended** as follows:

1. **(Once amended)** An encapsulation vesicle [for an assay], comprising:
  - (a) a matrix having a surface;
  - (b) a surface coating on said matrix, wherein said surface coating includes a fluorescent donor molecule that is absorbed on, absorbed within, or covalently attached to the surface of said matrix; and
  - (c) a protection layer encapsulating said surface coating, wherein upon irradiation said fluorescent donor molecule emits fluorescence that is at least partially transmitted through said surface coating, and wherein said protection layer reduces collisional quenching of said fluorescence [for protecting said surface coating from a quencher molecule; and
  - (d) a ligand attached to said protection layer].
2. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said matrix [is] comprises a sol-gel material.
3. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said matrix [surface] comprises silica and synthetic polymer.
4. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent donor molecule is an organo-metallic complex, and wherein the matrix surface is modified with carboxyl groups so that the organo-metallic complex[es] can be covalently attached to the matrix surface.

5. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent donor molecule is an organo-metallic complex, and wherein the matrix surface is modified with amino groups so that the organo-metallic complex[es] can be covalently attached to the matrix surface.
8. **(Once amended)** An encapsulation vesicle as recited in claim [7] 1, wherein said fluorescent donor molecule is an organo-metallic [material] complex.
10. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein said organo-metallic [material] complex is a ruthenium tris diphenyl phenanthroline complex.
11. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein said organo-metallic [material] complex has an emission maximum at about 650 nm.
13. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein said fluorescent donor molecule is selected from the group consisting of:



where Ln [=] is selected from the group consisting of Eu, Tb, Sm, and Dy[,]; and R represents H or a functionality capable of covalently linking to [a] the surface of said matrix.

14. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein [the] said fluorescent donor molecule [is any molecule having] has a fluorescence lifetime greater than 100 nanoseconds and [the molecules are] is susceptible to collisional quenching by oxygen.
15. **(Once amended)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a [translucent] material that is translucent to said fluorescence.
16. **(Once amended)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a [transparent] material that is transparent to said fluorescence.
17. **(Once amended)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a sol-gel [(silica) and synthetic polymers] material.
20. **(Once amended)** An encapsulation vesicle as recited in claim [1] 32, wherein said ligand [attached to said protection layer further] comprises an acceptor molecule that is capable of [receiving] absorbing fluorescence [energy transfer] that has been emitted from said fluorescent donor molecule [of said surface coating].
21. **(Once amended)** An encapsulation vesicle as recited in claim 1[,] for use in a fluorescence energy transfer [wherein said assay is an] immunoassay.
23. **(Once amended)** An encapsulation vesicle as recited in claim [1] 20, wherein [the acceptor's absorption band overlaps with the emission band of the donor] an absorption band of said acceptor molecule overlaps with an emission band of said fluorescent donor molecule.

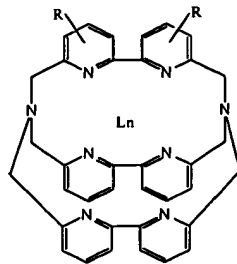
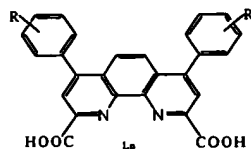
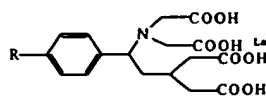
24. **(Once amended)** An encapsulation vesicle as recited in claim [1] 20, wherein [the] said acceptor molecule is selected from the group consisting of fluorescein, Cy5 and allophycocyanin.
25. **(Once amended)** An encapsulation vesicle as recited in claim [1] 32, wherein said ligand is an antibody.
26. **(Once amended)** An encapsulation vesicle as recited in claim [1] 21, wherein said fluorescence energy transfer immunoassay is a sandwich assay.
27. **(Once amended)** An encapsulation vesicle as recited in claim [1] 32, wherein [the biomolecule] said ligand is selected from the group consisting of proteins, DNA, RNA, polypeptides, aptamers and receptor molecules.

## APPENDIX C

### CLAIMS PENDING AFTER ENTRANCE OF AMENDMENT

1. **(Once amended)** An encapsulation vesicle, comprising:
  - (a) a matrix having a surface;
  - (b) a surface coating on said matrix, wherein said surface coating includes a fluorescent donor molecule that is absorbed on, absorbed within, or covalently attached to the surface of said matrix; and
  - (c) a protection layer encapsulating said surface coating, wherein upon irradiation said fluorescent donor molecule emits fluorescence that is at least partially transmitted through said surface coating, and wherein said protection layer reduces collisional quenching of said fluorescence.
2. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said matrix comprises a sol-gel material.
3. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said matrix comprises silica and synthetic polymer.
4. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent donor molecule is an organo-metallic complex, and wherein the matrix surface is modified with carboxyl groups so that the organo-metallic complex can be covalently attached to the matrix surface.
5. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent donor molecule is an organo-metallic complex, and wherein the matrix surface is modified with amino groups so that the organo-metallic complex can be covalently attached to the matrix surface.

6. **(Canceled)**
7. **(Canceled)**
8. **(Once amended)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent donor molecule is an organo-metallic complex.
9. **(Canceled)**
10. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein said organo-metallic complex is a ruthenium tris diphenyl phenanthroline complex.
11. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein said organo-metallic complex has an emission maximum at about 650 nm.
12. **(Canceled)**
13. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein said fluorescent donor molecule is selected from the group consisting of:



where  $L_n$  is selected from the group consisting of Eu, Tb, Sm, and Dy; and R represents H or a functionality capable of covalently linking to the surface of said matrix.

14. **(Once amended)** An encapsulation vesicle as recited in claim 8, wherein said fluorescent donor molecule has a fluorescence lifetime greater than 100 nanoseconds and is susceptible to collisional quenching by oxygen.
15. **(Once amended)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a material that is translucent to said fluorescence.
16. **(Once amended)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a material that is transparent to said fluorescence.
17. **(Once amended)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a sol-gel material.

18. **(Reiterated)** An encapsulation vesicle as recited in claim 2, wherein said protection layer is modified with hydrophilic functionalities selected from the group consisting of hydroxyl, carboxyl and protonated amines.
19. **(Reiterated)** An encapsulation vesicle as recited in claim 2 that was formed by suspension polymerization.
20. **(Once amended)** An encapsulation vesicle as recited in claim 37, wherein said ligand comprises an acceptor molecule that is capable of absorbing fluorescence that has been emitted from said fluorescent donor molecule.
21. **(Once amended)** An encapsulation vesicle as recited in claim 1 for use in a fluorescence energy transfer immunoassay.
22. **(Canceled)**
23. **(Once amended)** An encapsulation vesicle as recited in claim 20, wherein an absorption band of said acceptor molecule overlaps with an emission band of said fluorescent donor molecule.
24. **(Once amended)** An encapsulation vesicle as recited in claim 20, wherein said acceptor molecule is selected from the group consisting of fluorescein, Cy5 and allophycocyanin.
25. **(Once amended)** An encapsulation vesicle as recited in claim 37, wherein said ligand is an antibody.
26. **(Once amended)** An encapsulation vesicle as recited in claim 21, wherein said fluorescence energy transfer immunoassay is a sandwich assay.

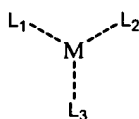


27. **(Once amended)** An encapsulation vesicle as recited in claim 37, wherein said ligand is selected from the group consisting of proteins, DNA, RNA, polypeptides, aptamers and receptor molecules.
28. **(Withdrawn)** A method of quantifying an analyte in a sample, comprising the steps of:
- (a) mixing a first binding molecule with a second binding molecule, wherein the first binding molecule competes with an analyte for binding the second binding molecule, wherein one of the first and second binding molecules is labeled with a photoluminescent energy transfer donor and the other is labeled with a photoluminescent energy transfer acceptor, wherein the photoluminescent energy transfer donor and acceptor are chosen such that when the first binding molecule binds to the second binding molecule, the donor and acceptor are brought into interacting proximity, producing a detectable luminescence change in the donor;
  - (b) encapsulating a second binding molecule;
  - (c) exposing the sample to an exciting amount of radiation;
  - (d) detecting the resulting emission; and
  - (e) calculating the apparent luminescence of the donor to quantify binding of the first binding molecule to the second binding molecule and thereby inversely quantifying the analyte.
29. **(Withdrawn)** The method of claim 29, wherein the photoluminescent donor is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, fluorescent infrared-emitting polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines and organo-metallic complexes.
30. **(Withdrawn)** The method of claim 29, where the photoluminescent acceptor is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines, organo-metallic complexes, and azo dyes.

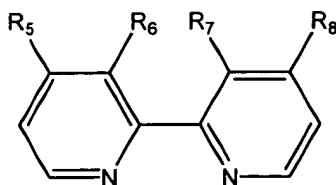
31. **(Withdrawn)** The sandwich method of quantifying an analyte in a sample, comprising the steps of:
- (a) mixing a first binding molecule with a second binding molecule, wherein the first binding molecule competes with an analyte for binding the second binding molecule, wherein one of the first and second binding molecules is labeled with a photoluminescent energy transfer donor and the other is labeled with a photoluminescent energy transfer acceptor, wherein the photoluminescent energy transfer donor and acceptor are chosen such that when the first binding molecule binds to the second binding molecule, the donor and acceptor are brought into interacting proximity, producing a detectable luminescence lifetime change in the photoluminescence lifetime of the donor;
  - (b) encapsulating a second binding molecule;
  - (c) exposing the sample to an exciting amount of radiation;
  - (d) detecting the resulting emission; and
  - (e) calculating the apparent luminescence lifetime of the donor without the use of a fluorescence intensity measurement to quantify the immune complex, thereby quantifying the analyte.
32. **(New)** An encapsulation vesicle as recited in claim 21, wherein said fluorescence energy transfer immunoassay is a competitive binding assay.
33. **(New)** An encapsulation vesicle as recited in claim 1 for use in a DNA or RNA fluorescence energy transfer hybridization assay.
34. **(New)** An encapsulation vesicle as recited in claim 1 for use in a fluorescence energy transfer binding assay between a ligand and a receptor.

35. **(New)** An encapsulation vesicle as recited in claim 34 for use in a fluorescence energy transfer binding assay between an aptamer and a protein.
36. **(New)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent donor molecule is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, fluorescent infrared-emitting polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines and organo-metallic complexes.
37. **(New)** An encapsulation vesicle as recited in claim 1 further comprising a ligand attached to said protection layer.
38. **(New)** An encapsulation vesicle as recited in claim 37, wherein said ligand is an antigen.
39. **(New)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises silica and synthetic polymer.
40. **(New)** An encapsulation vesicle as recited in claim 20, wherein said acceptor molecule is selected from the group consisting of Fast green and Light green SF yellowish.
41. **(New)** An encapsulation vesicle as recited in claim 20, wherein said acceptor molecule is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, fluorescent infrared-emitting polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines, organo-metallic complexes, and azo dyes.
42. **(New)** An encapsulation vesicle as recited in claim 1 further comprising an acceptor molecule attached to said protection layer, wherein said acceptor molecule is capable of absorbing fluorescence that has been emitted from said fluorescent donor molecule.
43. **(New)** An encapsulation vesicle as recited in claim 42, wherein said acceptor molecule is selected from the group consisting of Fast green and Light green SF yellowish.

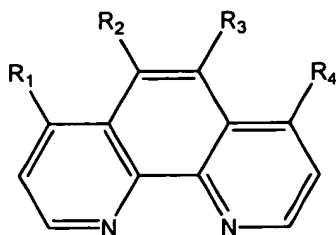
44. (New) An encapsulation vesicle as recited in claim 42, wherein said acceptor molecule is selected from the group consisting of fluorescein, Cy5 and allophycocyanin.
45. (New) An encapsulation vesicle as recited in claim 42, wherein said acceptor molecule is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, fluorescent infrared-emitting polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines, organo-metallic complexes, and azo dyes.
46. (New) An encapsulation vesicle as recited in claim 42, wherein an absorption band of said acceptor molecule overlaps with an emission band of said fluorescent donor molecule.
47. (New) An encapsulation vesicle as recited in claim 1, wherein said fluorescence is susceptible to collisional quenching by oxygen and said protection layer reduces the diffusion of oxygen into said surface coating.
48. (New) An encapsulation vesicle as recited in claim 8, wherein said fluorescent donor molecule is:



where M is selected from the group consisting of Ru, Os and Re; and  
 $L_1$ - $L_3$  are each independently selected from the group consisting of:

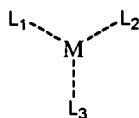


and



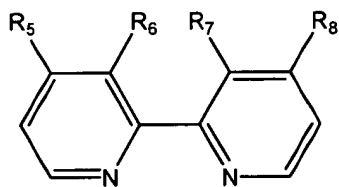
where R<sub>1</sub>-R<sub>8</sub> are each independently selected from the group consisting of H, alkyl and aryl.

49. (New) An encapsulation vesicle as recited in claim 8, wherein said fluorescent donor molecule is:

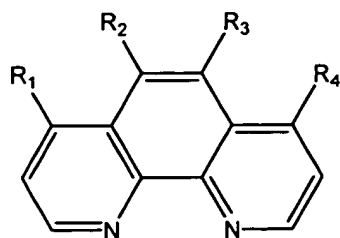


where M is Os;

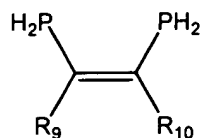
L<sub>1</sub> is:



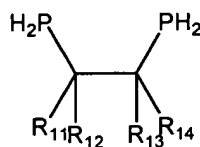
; or



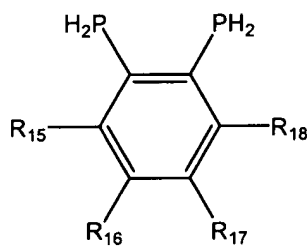
; and  $L_2$  and  $L_3$  are independently selected from the group consisting of:



;



; and



where  $R_1$ - $R_{18}$  are each independently selected from the group consisting of H, alkyl, and aryl.